Rocky Mountain Research Station

A protocol for collecting eDNA samples from snow tracks: field protocol (part 1)

Version 1.5, June 25, 2020

Collecting a snow sample from wolverine tracks in Montana, USA.

Authors:

Thomas W. Franklin, eDNA Program Leader, National Genomics Center for Wildlife and Fish Conservation, USDA Forest Service

Kevin S. McKelvey, Research Ecologist, National Genomics Center for Wildlife and Fish Conservation, USDA Forest Service

Jessie D. Golding, Carnivore Research Associate, National Genomics Center for Wildlife and Fish Conservation, USDA Forest Service

Justin J. Lex, Lead Biological Science Technician, National Genomics Center for Wildlife and Fish Conservation and Region 1, USDA Forest Service

Michael K. Schwartz, Director, National Genomics Center for Wildlife and Fish Conservation, USDA Forest Service

Table of Contents

Cite as Franklin, Thomas W., McKelvey, Kevin S., Golding, Jessie D., Lex, Justin J., and Schwartz, Michael K. 2020. A protocol for collecting eDNA samples from snow tracks: field protocol (part 1). U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

.

Introduction

Environmental DNA (eDNA) is DNA that has been released by an organism into the environment, such as air, water, or soil. Collection of eDNA was first used to describe microbial communities (Venter et al. 2004), but there has been an explosion of research on using eDNA sampling to detect macrobial species—fishes, amphibians, mollusks, crustaceans, and insects—in aquatic environments over the last decade (Thomsen and Willerslev 2015). Of particular interest has been using eDNA sampling to detect organisms that are rare or difficult to sample, whether they are invasive nonnative species (e.g., Dejean and others 2012; Goldberg and others 2013; Moyer and others 2014) or native species of conservation concern (Thomsen and others 2012; Wilcox and others 2013; Spear and others 2014).

In many instances, eDNA sampling has proven to be as or more effective than conventional sampling for determining presence, yet can often be performed more rapidly and efficiently (Dejean and others 2012; McKelvey and others 2016; Wilcox and others 2016). While these technologies have been used extensively to detect aquatic organisms, terrestrial systems have received much less attention.

In most boreal and temperate forests, noninvasive carnivore survey methods conducted in winter offer many advantages. For example, carnivore snow-tracks are common and easily located in winter. Canada lynx (*Lynx canadensis*) travel 1-9 km per day, leaving large numbers of tracks which can result in a 95% probability of detecting a Canada lynx if it is present given appropriate snow conditions and survey design (Squires and others 2004; 2012). However, winter noninvasive surveys for rare carnivores present many challenges. Noninvasive methods, like all wildlife survey methods, are subject to two major detection errors: false positives (species misidentifications) and false negatives (missed detections). Snow-track surveys are particularly vulnerable to misidentifications (e. g. Heinemeyer and others, 2008, Box 3.1, Clare and others 2017). Some species, such as fisher (*Pekania pennanti*) and marten (*Martes caurina* or *M. americana*), cannot be reliably separated via snow- tracks (Zielinski and Truex, 1995; Zielinski and others, 2006), leading to high levels of misidentification (Aubry and others, 2017; Aubry and Lewis, 2003; Clare and others 2017). Further, snow track surveys that use visual track identification can only occur when conditions are optimal; periods of frequent snowfall or melt are seldom conducive to accurate track identifications. To reduce track survey misidentification, surveyors use backtracking to locate genetic samples (i.e., scats or daybeds) (McKelvey and others 2006, Ulizio and others 2006). This can often require snowshoeing many kilometers off trail in un-compacted snow (e.g., Squires and others 2012).

As a potential alternative to backtracking, Dalén and others (2007) extracted DNA from arctic fox (*Alopex lagopus*) tracks using conventional PCR techniques typically applied to scat and hair samples, but had limited success (16.7%, 1/6). These low success rates were deemed unsatisfactory, and the method was not pursued. The quantitative PCR

methods used for collection of eDNA are at least an order of magnitude more sensitive than conventional PCR; in laboratory tests single copies of DNA are generally detected and 10+ copies of DNA are detected >95% of the time (e. g. Wilcox and others 2013). Franklin and others (2019) therefore tested qPCR approaches on snow tracks for 3 species: Canada lynx, fisher, and wolverine (*Gulo gulo*). Rates of detection were 100% for lynx (11/11) and fisher (3/3) and 76% (13/17) for wolverines. The wolverine detection rate is likely conservative; we were unable to ascertain for certain that all putative wolverine tracks were, in fact, made by wolverines. Given this high success rate, DNA identification from snow tracks is both much easier and more reliable than backtracking. McKelvey and others (2006) found that for lynx, backtracking 1 km will produce a usable DNA sample about 40% of the time. We are currently (Fall 2018) developing assays for bobcat (*Lynx rufus*), cougar (*Puma concolor*), coyote (*Canis latrans*), grizzly bear (*Ursus arctos*), marten, and red fox (*Vulpes vulpes*).

Because identifying tracks from snow samples can be done under a wide variety of snow conditions, and because it has a high success rate with trivial rates of misidentification, we are currently prototyping it with the intention of making it a major component of winter carnivore surveys.

Below is a protocol for collecting snow samples and pre-processing them to the point that they can be sent to the National Genomics Center for Wildlife and Fish Conservation for species identification. The protocol is based on Carim et al. (2016) a field protocol for sampling aquatic systems.

Before heading to the field

Because of the extreme sensitivity of the assays, it is critical that snow tracks be properly collected to both increase the chance of detecting the target organism, and to minimize the potential for contamination. Further, the efficacy of the technique is dependent on both how snow samples are collected and the overall water content of the snow sample. The approach here closely follows testing in Franklin and others (2019); we know that samples collected in this manner will generally have sufficient DNA for analysis and will be free from contamination. It is therefore essential to follow this protocol to ensure both reliability and efficacy.

Three points:

1. The kit described below contains all of the materials needed to collect eDNA. You will need to provide a GPS unit or GPS-equipped device to determine the sampling location.

2. Once collected, snow samples should be kept frozen. If outdoor temperatures remain below freezing, the shady side of a building may be sufficient. Do not store in a freezer that has contained any of the target species at any time.

3. Once samples are melted they should filtered quickly using following the protocol below.

The eDNA sampling kit

Each sample is collected using a pre-built kit that includes all components. We assemble these and distribute them to collaborators to ensure that the components have been tested for cleanliness and to standardize the sampling process. However, we are aware that many of the most important track locations for rare carnivores will be in unusual areas on the periphery of the species' range rather than collected as part of an organized survey. If all components are new and the kits are assembled in areas and by personnel who have had no contact with the target organism, these kits can be safely constructed and used to identify tracks.

Figure 1. The eDNA field sampling kit

Each individual sample kit contains:

1. Plastic Scoop for collecting snow
2. A 2 l Whirl-pac
3. Pair of elbow length food service gloves
4. An 8 l (2 gal) Ziploc bag
5. A Sharpie
6. A 4 l (1 gal) Ziploc bag to hold the other components prior to sampling.

Groups of individual sample bags will be enclosed inside of a white plastic garbage sack (Figure 1), which helps them to stay clean (though the outside of the 4 l bags containing the sample kits will be considered dirty). Also included is a black trash bag to put the used materials (gloves, scoop, Ziploc bag, Sharpie) in and (depending on the number of samples), several white plastic trash bags to hold samples once collected. We reuse the scoops and the sharpies.

Clean procedures and avoiding contamination

The most important thing you can do to ensure the accuracy of your eDNA results is to avoid contamination of the field sample. The primary sources of contamination are anything that may have come in contact with the target species: hands, clothes, snowmobiles, snowshoes etc. The easiest way to think about keeping the sample clean is to think in terms of things that are from the site (clean) and things that have been transported into the site (potentially dirty). So the snow, or the trees, logs etc. that are part of the environment are clean—the environment is what you are sampling. With the exception of the kit elements inside of the 4 l Ziploc bag, everything else that you brought to the site is considered to be dirty. This protocol has been designed to allow you to collect a sample while keeping the sample isolated from potentially contaminating clothing and materials.

Field collection protocol

Samples from each monitoring unit should be grouped into a white plastic trash bag—bags are provided. Before you collect the first sample in an area, take out one of the white bags and write information concerning the area and the date. There should be enough information on the white bag that the unit can be relocated based on this information alone. Information should include the area label, a generalized descriptor of the area (e. g. “south Pioneer Mountains” or “Madison R.D.”, etc.), and your initials.

You will be taking 2 samples at each putative track location. This both improves our chances that DNA is collected and provides lack-of-detection statistics and measures of sample-to sample variance. You will, therefore repeat this process twice.

Step 1) Remove an individual sample kit from the white bag.

Step 2) Locate tracks. You will be sampling the compacted snow that came into contact with the animal. If the tracks are covered by deep snow, you may wish to follow the track line until it enters an area with less new snow, such as under a tree canopy. Sampled tracks should always be in front of you in areas where nobody from your crew has walked. If the track has an uphill direction, sample uphill—it’s easier to keep the snow you disturb away from the sample area.

Step 3) Open your kit. When you have reached the location you are going to sample, holding onto the sides of the 4 l Ziploc bag, pull it open. When open, use the bag to shuffle the gloves up to where they can be reached without touching the rest of the contents (depending on snow conditions, you may find it easier to dump the contents of the bag onto a “clean” surface such as untracked snow).

Step 4) Put the gloves on. You will need to rub the cuff between your fingers to open the gloves up. Do this for both gloves with an un-gloved hand. If you are right handed, put the left glove on first, if left handed, put the right glove on first. The gloves should be pulled up to or above the elbow. They are loose fitting and should allow fleece and lighter jackets (e. g. down sweaters) and liner gloves to be kept on through the sampling process, but heavy gloves and thick jackets will likely need to be removed prior to sampling.

Step 5) Label the Whirl-pac with the sharpie. Include:

- Your initials
- Monitoring unit label if pertinent
- Area name (e. g. “Grouse Creek”)
- A unique identifier and “A” for the first sample and “B” for the second sample. This identifier should be the same on both samples. (e. g. GC 137-A and GC 137-B)
- UTM of the sampling location; including zone
- Date
- Putative species—the species you believe made the tracks
- 

Step 6) Open the Whirl-pac. To open, rip off the top strip and, pull on the little white tabs and/or push in on the ends of the stiff strip.

Step 7) Scoop up as many prints as will fit in the Whirl-pac; pack them in, but leave enough space to close the Whirl-pac. Try to **scoop as much of the compacted areas associated with the track as possible**. Even in powdery snow, there should be "stiffer" areas created by the animal's passage. **The goal is to get as much snow that would have touched the organism's feet as possible.** Use the scoop to move excess snow that has fallen after the animal created the track if necessary.

Step 8) Close the Whirl-pac. Pull on the ends of the stiff strip to close it and, holding onto the ends at arms-length spin the Whirl-pac 3 complete revolutions and bend in the ends in to complete the seal. Caution, the gloves can make this slippery. Alternatively, you can roll the top 3 times, making sure to keep the ends tight.



Set the Whirl-pac in a clean place (e. g. on untracked snow). Pick up the 8 l bag that was inside the sample kit, open it, and place the sample bag inside, squeezing as much air out as practical prior to sealing it. Put the scoop, 4 l Ziploc bag and sharpie and any other trash into the black trash bag. Remove the gloves and put them in the black bag as well. You are done with a sample. Place the sample in one of the white trash bags for storage.

NOTE: **Keep the scoop and sharpies.** We clean and reuse the sharpies and the scoops.

Control samples

You will periodically collect control samples. These are samples from the environment away from tracks. The procedure is identical to that associated with taking a track sample; the only difference is where the sample is taken. The rules for control samples are to take **1 sample for every 5 pairs of track samples**, or **take 1 sample per day in the field**. That is, you will always collect at least 1 control sample for each day in the field. Collect these samples throughout the day—don't collect them all at the beginning or end. If you believe you will likely only collect 1 control sample, collect it towards the middle of the day and after you have collected at least 1 track sample. If you are anticipating only collecting a single sample (such as when someone tells you that they have seen a track at a specific location and you are going to check out the report), collect the control sample soon after collecting the track sample.

Control samples should be collected from untracked surface snow at least 100m from any putative target tracks. Don't sample deeper snow: it may have been tracked earlier in the season. Skim the surface until you have filled the Whirl-pac. Please label the control samples in the same way you label the track samples, except that the "putative track sample" should be filled in with the word **CONTROL**.

Emergency kits

You may desire to collect a track sample, but not have access to a kit. You can make a reasonable kit with no other supplies than a new box of 4 l (1 gal.) Ziploc or similar bags and a new sharpie. As with the official kit, you will be collecting 2 samples, and should follow the field protocol above when choosing where to sample. Open the box and remove 2 bags. These will serve as gloves. Open the Sharpie packaging. With the bags on as gloves, remove another 2 bags, set one aside on the snow. On the other, write your name, the general area name (e. g. "Grouse Creek"), a unique identifier and "A" or "B", the UTM including zone of the sampling location, the date, and the putative species—the species you believe made the tracks. Put the sharpie somewhere out of the way and open the bag you just wrote on. The open bag will serve to hold the sample. With your hands inside the Ziploc bags separate the compacted track snow and place it in the open Ziploc. When about ½ full of packed snow, seal the bag and place it inside of the second bag that you set-aside. Because this method is a bit clumsy and the bags aren't as effective as the shoulder-length gloves for keeping you separated from the sample, always collect a control sample when using this emergency system.

After sampling, transportation and on-site filtering

In the field, if it is constantly below freezing (freeze – thaw cycles can severely harm DNA), bags of samples can be kept outside, for example on the north side of a building that is not exposed to UV light (direct sunlight), until you are ready to transport them. If you use a freezer for storage, be sure that it has never contained any tissue either from the target species or for other species that may have made the track.

Depending on circumstances and prior arrangements samples can be filtered remotely and the filters shipped rather than the snow samples. This approach requires following stringent guidelines and additional equipment, which can be obtained from the National Genomics Center. See: A protocol for collecting eDNA samples from snow tracks: filtration protocol (part 2).

If snow samples are to be shipped, keep them in the white trash bags throughout storage and shipping both to ensure that geographically related samples are kept together and to reduce the possibility of contamination. During ground transportation, in many cases a cooler will provide a sufficient container; a large group of samples represents a significant mass of snow that should remain frozen in a cooler for several days. Single, or a few, samples that are mailed should be packaged with dry ice in an insulated

container and shipped overnight as they have a much greater opportunity to melt. Samples should not be allowed to melt until immediately prior to filtration. See: A protocol for collecting eDNA samples from snow tracks: filtration protocol (part 2).

All samples should be sent to:

Tommy Franklin, eDNA Program Leader
800 East Beckwith Ave.
Missoula, MT 59801
406-542-4171
thomas.franklin@usda.gov

For questions regarding the Multispecies Mesocarnivore Monitoring Program contact:

Jessie Golding, Multispecies Mesocarnivore Monitoring Program Leader
800 East Beckwith Ave.
Missoula, MT 59801
406-542-4158
jessie.golding@usda.gov

References

Aubry KB, Lewis JC (2003) Extirpation and reintroduction of fishers (Martes pennanti) in Oregon: Implications for their conservation in the Pacific states. Biological Conservation, 114, 79–90. https://doi.org/10.1016/S0006-3207(03)00003-X

Aubry KB, Raley CM, McKelvey KS (2017). The importance of data quality for generating reliable distribution models for rare, elusive, and cryptic species. PLoS One 12, e0179152. https://doi.org/10.1371/journal.pone.0179152

Barnes MA, Turner CR (2016). The ecology of environmental DNA and implications for conservation genetics. Conservation Genetics, 17, 1–17.

Clare J, McKinney ST, DePue JE, Loftin CS (2017) Pairing field methods to improve inference in wildlife surveys while accommodating detection covariance. Ecological Applications, 27, 2031-2047. https://doi.org/10.1002/eap.1587

Dalén L, Götherström A, Meijer T, Shapiro B (2007) Recovery of DNA from footprints in the snow. Canadian Field-Naturalist, 121, 321-324. https://doi.org/10.22621/cfn.v121i3.482

Dejean T, Valentini A, Miquel C, Taberlet P, Bellemain E, Miaud C (2012) Improved detection of an alien invasive species through environmental DNA barcoding: the

example of the American bullfrog Lithobates catesbeianus. Journal of Applied Ecology, 49, 953–959.

Franklin TW, McKelvey KS, Golding JD, Mason DH, Dysthe JC, Pilgrim KL, Squires JR, Aubry KB, Long RA, Greaves SE, Raley CM, Jackson S, Mackay P, Lisbon J, Sauder JD, Pruss MT, Heffington D, Schwartz MK (2019) Using environmental DNA methods to improve winter surveys for rare carnivores: DNA from snow and improved noninvasive techniques. Biological Conservation, 229, 50-58. https://doi.org/10.1016/j.biocon.2018.11.006

Goldberg CS, Sepulveda A, Ray A, Baumgardt J, Waits LP (2013) Environmental DNA as a new method for early detection of New Zealand mudsnails (Potamopyrgus antipodarum). Freshwater Science, 32, 792–800.

Heinemeyer KS, Ulizio T, Harrison RL, Long RA, MacKay P, (2008) Natural sign: tracks and scats, in: Noninvasive Survey Methods for Carnivores. Island Press, Washington D.C., USA, pp. 45–74.

McKelvey KS, Von Kienast J, Aubry KB, Koehler GM, Maletzke BT, Squires JR, Lindquist EL, Loch S, Schwartz MK (2006) DNA analysis of hair and scat collected along snow tracks to document the presence of Canada lynx. Wildlife Society Bulletin, 34, 451–455. https://doi.org/10.2193/0091-7648(2006)34[451:DAOHAS]2.0.CO;2

McKelvey KS, Young MK, Knotek WL, Carim KJ, Wilcox TM, Padgett-Stewart TM, Schwartz MK (2016) Sampling large geographic areas for rare species using environmental DNA: A study of bull trout Salvelinus confluentus occupancy in western Montana. Journal of Fish Biology, 88, 1215–1222. https://doi.org/10.1111/jfb.12863

Moyer GR, Díaz-Ferguson E, Hill JE, Shea C (2014) Assessing environmental DNA detection in controlled lentic systems. PLoS ONE, 9, e103767. doi:10.1371/journal.pone.0103767

Squires, J.R., McKelvey, K.S., Ruggiero, L.F., 2004. A snow-tracking protocol used to delineate local lynx, Lynx canadensis, distributions. Can. Field-Naturalist 118. https://doi.org/10.22621/cfn.v118i4.60

Squires, J.R., Olson, L.E., Turner, D.L., Decesare, N.J., Kolbe, J.A., 2012. Estimating detection probability for Canada lynx Lynx canadensis using snow-track surveys in the northern Rocky Mountains, Montana, USA. Wildlife Biol. 18, 215–224. https://doi.org/10.2981/10-105

Spear SF, Groves JD, Williams LA, Waits LP (2014) Using environmental DNA methods to improve detectability in a hellbender (Cryptobranchus alleganiensis) monitoring program. Biological Conservation. doi:10.1016/j.biocon.2014.11.016

Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L, Willerslev E (2012) Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology, 21, 2565–2573. doi:10.1111/j.1365-294X.2011.05418.x

Thomsen PF, Willerslev E (2015) Environmental DNA – An emerging tool in conservation monitoring past and present. Biological Conservation, 183, 4–18.

Ulizio TJ, Squires JR, Pletscher DH, Schwartz MK, Claar JJ, Ruggiero LF (2006) The efficacy of obtaining genetic-based identifications from putative wolverine snow tracks. Wildlife Society Bulletin, 34, 1326–1332. https://doi.org/10.2193/0091-7648(2006)34[1326:TEOOGI]2.0.CO;2

Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., Wu, D., Paulsen, I., Nelson, K.E., Nelson, W. and Fouts, D.E. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. Science, 304, 66-74.

Wilcox TM, McKelvey KS, Young MK, Jane SF, Lowe WH, Whiteley AR, Schwartz MK (2013) Robust detection of rare species using environmental DNA: the importance of primer specificity. PLoS ONE, 8, e59520. doi: 10.1371/journal.pone.0059520

Wilcox TM, McKelvey KS, Young MK, Sepulveda AJ, Shepard BB, Jane SF, Whiteley AR, Lowe WH, Schwartz MK (2016) Understanding environmental DNA detection probabilities: a case study using a stream-dwelling char Salvelinus fontinalis. Biological Conservation, 194, 209–216.

Zielinski WJ, Schlexer FV, Pilgrim KL, Schwartz MK (2006) The Efficacy of Wire and Glue Hair Snares in Identifying Mesocarnivores. Wildlife Society Bulletin, 34, 1153–1161. https://doi.org/10.1111/j.1365-3040.1991.tb01380.x

Zielinski WJ, Truex RL (1995) Distinguishing Tracks of Marten and Fisher at Track-Plate Stations. Journal of Wildlife Management, 59, 571-579. https://doi.org/10.2307/3802465